

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as shown:

Please delete the paragraph on page 6, lines 11-14,
and replace it with the following paragraph:

(3) $V_1S_2G_3G_4S_5I_6S_7<1>_8<1>_9<1>_{10}Y_{11}Y_{12}W_{13}<1>_{14}$

(SEQ ID NO: 1), wherein <1> is an equimolar mixture of
each of amino acid residues A, D, E, F, G, H, I, K, L, M,
N, P, Q, R, S, T, V, W, and Y; and

Please delete the paragraph on page 6, line 27,
through page 7, line 2, and replace it with the following
paragraph:

(1) $<2>I<2><3>SGG<1>T<1>YADSVKG$ (SEQ ID
NO: 2), wherein <1> is an equimolar mixture of each of
amino acid residues A, D, E, F, G, H, I, K, L, M, N, P,
Q, R, S, T, V, W, and Y; <2> is an equimolar mixture of
each of amino acid residues Y, R, W, V, G, and S; and <3>
is an equimolar mixture of each of amino acid residues P,
S, and G or an equimolar mixture of P and S;

Please delete the paragraph on page 7, lines 3-8,
and replace it with the following paragraph:

(2) $<1>I<4><1><1><G><5><1><1><1>YADSVKG$
(SEQ ID NO: 3), wherein <1> is an equimolar mixture of
each of amino acid residues A, D, E, F, G, H, I, K, L, M,

N, P, Q, R, S, T, V, W, and Y; <4> is an equimolar mixture of residues D, I, N, S, W, Y; and <5> is an equimolar mixture of residues S, G, D and N;

Please delete the paragraph on page 7, lines 9-12, and replace it with the following paragraph:

(3) <1>I<4><1><1>G<5><1><1>YNPSLKG (SEQ ID NO: 4), wherein <1> is an equimolar mixture of each of amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and <4> and <5> are as defined above;

Please delete the paragraph on page 7, lines 13-17, and replace it with the following paragraph:

(4) <1>I<8>S<1><1><1>GGYY<1>YAASVKG (SEQ ID NO: 5), wherein <1> is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; <8> is 0.27 R and 0.027 of each of ADEFGHIKLMNPQSTVWY; and

Please delete the paragraph on page 7, line 31, through page 8, line 2, and replace it with the following paragraph:

(1) YYCA21111YFDYWG (SEQ ID NO: 6), wherein 1 is an equimolar mixture of each amino acid

residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

Please delete the paragraph on page 8, and replace it with the following page:

(2) YYCA2111111YFDYWG (SEQ ID NO: 7), wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(3) YYCA21111111YFDYWG (SEQ ID NO: 8), wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(4) YYCAR111S2S3111YFDYWG (SEQ ID NO: 9), wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of S and G; and 3 is an equimolar mixture of Y and W;

(5) YYCA2111CSG11CY1YFDYWG (SEQ ID NO: 10), wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(6) YYCA211S1TIFG11111YFDYWG (SEQ ID NO: 11), wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(7) YYCAR111YY2S3344111YFDYWG (SEQ ID NO: 12), wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; 2 is an equimolar mixture of D and G; and 3 is an equimolar mixture of S and G;

(8) YYCAR1111YC2231CY111YFDYWG (SEQ ID NO: 13), wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; 2 is an equimolar mixture of S and G; and 3 is an equimolar mixture of T, D and G; and

Please delete the paragraph on page 9, line 26, through page 10, line 11, and replace it with the following paragraph:

4. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by

variegated DNA sequences that encodes a kappa light chain
CDR1 selected from the group consisting of:

(1) RASQ<1>V<2><2><3>LA (SEQ ID NO: 14)

(2) RASQ<1>V<2><2><2><3>LA (SEQ ID NO:
15);

wherein <1> is an equimolar mixture of amino acid
residues ADEFGHIKLMNPQRSTVWY; <2> is 0.2 S and 0.044 of
each of ADEFGHIKLMNPQRTVWY; and <3> is 0.2Y and 0.044
each of ADEFGHIKLMNPQRTVW and Y; and

(3) mixtures of vectors or genetic
packages characterized by any of the above DNA sequences,
preferably in the ratio CDR1s (1):(2)::0.68:0.32.

Please delete the paragraph on page 10, line 26, to
page 11, line 15, and replace it with the following
paragraph:

6. A focused library of vectors or genetic
packages that display, display and express, or comprise a
member of a diverse family of human antibody related
peptides, polypeptides and proteins and collectively
display, display and express, or comprise at least a
portion of the diversity of the antibody family, the
vectors or genetic packages being characterized by

variegated DNA sequences that encode a kappa light chain
CDR3 selected from the groups consisting of:

(1) QQ<3><1><1><1>P<1>T (SEQ ID NO: 16),

wherein <1> is an equimolar mixture of amino acid
residues ADEFGHIKLMNPQRSTVWY; <3> is 0.2 Y and 0.044 each
of ADEFGHIKLMNPQRTVW;

(2) QQ33111P, wherein 1 and 3 are as
defined in (1) above;

(3) QQ3211PP1T (SEQ ID NO: 17), wherein 1
and 3 are as defined in (1) above and 2 is 0.2 S and
0.044 each of ADEFGHIKLMNPQRTVWY; and

(4) mixtures of vectors or genetic
packages characterized by any of the above DNA sequences,
preferably in the ratio CDR3s (1):(2):(3)::0.65:0.1:0.25.

Please delete the paragraph on page 11, line 16,
through page 12, line 6, and replace it with the
following paragraph:

7. A focused library of vectors or genetic
packages that display, display and express, or comprise a
member of a diverse family of human antibody related
peptides, polypeptides and proteins and collectively
display, display and express, or comprise at least a

portion of the diversity of the antibody family, the vectors or genetic packages being characterized by variegated DNA sequences that encode a lambda light chain CDR1 selected from the group consisting of:

(1) TG<1>SS<2>VG<1><3><2><3>VS (SEQ ID NO: 18), wherein <1> is 0.27 T, 0.27 G and 0.027 each of ADEFHIKLMNPQRSVWY, <2> is 0.27 D, 0.27 N and 0.027 each of AEFGHIKLMNPQRSTVWY, and <3> is 0.36 Y and 0.036 each of ADEFHIKLMNPQRSTVW;

(2) G<2><4>L<4><4><4><3><4><4>,

wherein <2> is as defined in (1) above and <4> is an equimolar mixture of amino acid residues ADEFHIKLMNPQRSTVWY; and

(3) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio CDR1s (1):(2)::0.67:0.33.

Please delete the paragraph on page 12, line 20, through page 13, line 8, and replace it with the following paragraph:

9. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively

display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by variegated DNA sequences that encode a lambda light chain CDR3 selected from the group consisting of:

(1) $\langle 4 \rangle \langle 5 \rangle \langle 4 \rangle \langle 2 \rangle \langle 4 \rangle S \langle 4 \rangle \langle 4 \rangle \langle 4 \rangle V$, wherein $\langle 2 \rangle$ is 0.27 D, 0.27 N, and 0.027 each of A E F G H I K L M P Q R S T V W Y; $\langle 4 \rangle$ is an equimolar mixture of amino acid residues A D E F G H I K L M N P Q R S T V W; and $\langle 5 \rangle$ is 0.36 S and 0.0355 each of A D E F G H I K L M N P Q R T V W Y;

(2) $\langle 5 \rangle S Y \langle 1 \rangle \langle 5 \rangle S \langle 5 \rangle \langle 1 \rangle \langle 4 \rangle V$ (SEQ ID NO: 19), wherein $\langle 1 \rangle$ is an equimolar mixture of A D E F G H I K L M N P Q R S T V W Y; and $\langle 4 \rangle$ and $\langle 5 \rangle$ are as defined in (1) above; and

(3) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio CDR3s (1):(2)::1:1.

Please delete the paragraph on page 15, lines 13-16, and replace it with the following paragraph:

In addition, JH4 (YFDYWGGQTLVTUSS) (SEQ ID NO: 20) occurs more often than JH3 in native antibodies. Hence, it is preferred for the focused libraries of this

invention. However, JH3 (AFDIWGQGTMTVSS) (SEQ ID NO: 21) could as well be used.

Please delete the paragraph on page 18, lines 12-22, and replace it with the following paragraph:

The two less preferred HC CDR1s of this invention have length 7 and length 14. For length 7, a preferred variegation is

(S/T)₁(S/G/<1>)₂(S/G/<1>)₃Y₄Y₅W₆(S/G/<1>)₇; where (S/T)

indicates an equimolar mixture of Ser and Thr codons;

(S/G/<1>) indicates a mixture of 0.2025 S, 0.2025 G, and 0.035 for each of A, D, E, F, H, I, K, L, M, N, P, Q, R,

T, V, W, Y. This design gives a predominance of Ser and Gly at positions 2, 3, and 7, as occurs in mature HC

genes. For length 14, a preferred variegation is

VSGGSIS<1><1><1>YYW<1> (SEQ ID NO: 1), where <1> is an equimolar mixture of the 19 native amino acid residues, except Cys (C).

Please delete the paragraph on page 19, lines 23-32, and replace it with the following paragraph:

Diversity in HC CDR2 was designed with the same considerations as for HC CDR1: GLG sequences, mature sequences and 3D structure. A preferred length for CDR2 is 17, as shown in Table 1. For this preferred 17 length CDR2, the preferred variegation in accordance with the invention is: <2>I<2><3>SGG<1>T<1>YADSVKG (SEQ ID NO: 2),

where <2> indicates any amino acid residue selected from the group of Y, R, W, V, G and S (equimolar mixture), <3> is P, S and G or P and S only (equimolar mixture), and <1> is any native amino acid residue except C (equimolar mixture).

Please delete the paragraph on page 20, lines 9-17, and replace it with the following paragraph:

In an alternative embodiment for a 17 length HC CDR2, the following variegation may be used:

<1>I<4><1><1>G<5><1><1><1>YADSVKG (SEQ ID NO: 3), where <1> is as described above for the more preferred alternative of HC CDR2; <4> indicates an equimolar mixture of DINSWY, and <5> indicates an equimolar mixture of SGDN. This diversity pattern is embodied in ON-R2V2vg shown in Table 3. Preferably, the two embodiments are used in equimolar mixtures in the libraries of this invention.

Please delete the paragraph on page 20, lines 18-33, and replace it with the following paragraph:

Other preferred HC CDR2s have lengths 16 and 19. Length 16: <1>I<4><1><1>G<5><1><1>YNPSLKG (SEQ ID NO: 4); Length 19: <1>I<8>S<1><1><1>GGYY<1>YAASVKG (SEQ ID NO: 5), wherein <1> is an equimolar mixture of all native amino acid residues except C; <4> is a equimolar mixture of DINSWY; <5> is an equimolar mixture of SGDN; and <8>

is 0.27 R and 0.027 of each of residues

ADEFGHIKLMNPQSTVWY. Table 3 shows ON-R2V3vg which embodies a preferred CDR2 variegation of length 16 and ON-R2V4vg which embodies a preferred CDR2 variegation of length 19. To prepare these variegations ON-R2V3vg may be PCR amplified with ON-R2top and ON-R2bo3 and ON-R2V4vg may be PCR amplified with ON-R2top and ON-R2-bo4. See Table 3. In the most preferred embodiment of this invention, all three HC CDR2 lengths are used. Preferably, they are present in a ratio 17:16:19::579:464:31::0.54:0.43:0.03.

Please delete the paragraph on page 21, lines 19-32, and replace it with the following paragraph:

Component 1 represents all the genes having lengths 0 to 8 (counting from the YYCAR (SEQ ID NO: 22) motif at the end of FR3 to the WG dipeptide motif near the start of the J region, i.e., FR4). Component 2 corresponds to all the genes having lengths 9 or 10. Component 3 corresponds to the genes having lengths 11 or 12 plus half the genes having length 13. Component 4 corresponds to those having length 14 plus half those having length 13. Component 5 corresponds to the genes having length 15 and half of those having length 16. Component 6 corresponds to genes of length 17 plus half of those with length 16. Component 7 corresponds to

those with length 18. Component 8 corresponds to those having length 19 and greater. See Table 4.

Please delete the paragraph on page 23, line 31, to page 24, line 14, and replace it with the following paragraph:

In native human kappa chains, CDR1s with lengths of 11, 12, 13, 16, and 17 were observed with length 11 being predominant and length 12 being well represented. Thus, in the preferred embodiments of this invention LC CDR1s of length 11 and 12 are used in an and mixture similar to that observed in native antibodies), length 11 being most preferred. Length 11 has the following sequence: RASQ<1>V<2><2><3>LA (SEQ ID NO: 14) and Length 12 has the following sequence: RASQ<1>V<2><2><2><3>LA (SEQ ID NO: 15), wherein <1> is an equimolar mixture of all of the native amino acid residues, except C, <2> is 0.2 S and 0.044 of each of ADEFGHIKLMNPQRTVWY, and <3> is 0.2 Y and 0.044 each of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y. In the most preferred embodiment of this invention, both CDR1 lengths are used. Preferably, they are present in a ratio of 11:12::154:73::0.68:0.32.

Please delete the paragraph on page 24, line 24, through page 25, line 3, and replace it with the following paragraph:

In native kappa, CDR3 exhibits lengths of 1, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 19. While any of these lengths and mixtures of them can be employed in this invention, we prefer lengths 8, 9 and 10, length 9 being more preferred. For the preferred Length 9, the sequence is QQ<3><1><1><1>P<1>T (SEQ ID NO: 16), wherein <1> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY and <3> is 0.2 Y and 0.044 each of ADEFGHIKLMNPQRSVW. Length 8 is preferably QQ33111P and Length 10 is preferably QQ3211PP1T (SEQ ID NO: 17), wherein 1 and 3 are as defined for Length 9 and 2 is S (0.2) and 0.044 each of ADEFGHIKLMNPQRTVWY. A mixture of all 3 lengths being most preferred (ratios as in native antibodies), i.e., 8:9:10::28:166:63::0.1:0.65:0.25.

Please delete the paragraph on page 26, lines 6-18, and replace it with the following paragraph:

In native human lambda chains, CDR1s with length 14 predominate, lengths 11, 12 and 13 also occur. While any of these can be used in this invention, lengths 11 and 14 are preferred. For length 11 the sequence is: TG<2><4>L<4><4><4><3><4><4> and for Length 14 the sequence is: TG<1>SS<2>VG<1><3><2><3>VS (SEQ ID NO: 18), wherein <1> is 0.27 T, 0.27 G and 0.027 each of ADEFHIKLMNPQRSVWY; <2> is 0.27 D, 0.27 N and 0.027 each of AEFGHIKLMPQRSTVWY; <3> is 0.36 Y and 0.0355 each of

ADEFGHIKLMNPQRSTVW; and <4> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY. Most preferably, mixtures (similar to those occurring in native antibodies) preferably, the ratio is 11:14::23:46::0.33: 0.67 of the three lengths are used.

Please delete the paragraph on page 26, line 27, through page 27, line 7, and replace it with the following paragraph:

In native human lambda chains, CDR3s of length 10 and 11 predominate, while length 9 is also common. Any of these three lengths can be used in the invention. Length 11 is preferred and mixtures of 10 and 11 more preferred. The sequence of Length 11 is <4><5><4><2><4>S<4><4><4><4>V, where <2> and <4> are as defined for the lambda CDR1 and <5> is 0.36 S and 0.0355 each of ADFFGHIKLMNPQRTVWY. The sequence of Length 10 is <5>SY<1><5>S<5><1><4>V (SEQ ID NO: 19), wherein <1> is an equimolar mixture of ADEFGHIKLMNPQRSTVWY; and <4> and <5> are as defined for Length 11. The preferred mixtures of this invention comprise an equimolar mixture of Length 10 and Length 11. Table 8 shows a preferred focused lambda light chain diversity in accordance with this invention.

Please delete Tables 1-11 on pages 30-74, and replace with the following Tables:

Table 1: 3-23:JH4 CDR1/2 diversity = 1.78×10^8
(SEQ ID NOS 23-24 are nucleotide and encoded amino acid, respectively)

| | | | | | | | | | | | | | | | | | | | | | | |
|----------------------------|--|--|--|--|--|--|--|--|--|---------------------------------|-----|-----|--|--|--|--|--|--|--|--|--|--|
| | | | | | | | | | | FR1 (VP47/V3-23) ----- | | | | | | | | | | | | |
| | | | | | | | | | | 20 | 21 | 22 | | | | | | | | | | |
| | | | | | | | | | | A | M | A | | | | | | | | | | |
| ctgtctgaac (SEQ ID NO: 99) | | | | | | | | | | cc | atg | gcc | | | | | | | | | | |
| Scab..... | | | | | | | | | | NcoI..... | | | | | | | | | | | | |
| | | | | | | | | | | MfeI | | | | | | | | | | | | |
| | | | | | | | | | | gaa ggt caa ttg tta gag tct ggt | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------------------------------------------------|----|----|----|----|----|----|----|----|----|---------------|-----|-----|-----|-----|---------------------|--|--|--|--|--|--|--|--|--|--|
| -----FR1----- | | | | | | | | | | -----FR2----- | | | | | | | | | | | | | | | |
| 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 6859-fold diversity | | | | | | | | | | |
| G G L V Q P G G S L R L S C A | | | | | | | | | | | | | | | | | | | | | | | | | |
| ggc ggt ctt ggt cag cct ggt ggt tct tta cgt ctt tct tgc gct | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sites of variegation | | | | | | | | | | <1> | <1> | <1> | <1> | <1> | <1> | | | | | | | | | | |
| -----FR1----->CDR1..... -----FR2----- | | | | | | | | | | | | | | | | | | | | | | | | | |
| 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | | | | | | | | | | | |
| A S G F T F S - Y - M - W V R | | | | | | | | | | | | | | | | | | | | | | | | | |
| gct tcc gga ttc act ttc tct - tac - atg - tgg ggt cgc | | | | | | | | | | | | | | | | | | | | | | | | | |
| BspEI | | | | | | | | | | BsiWI | | | | | | | | | | | | | | | |
| | | | | | | | | | | BstXI. | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | | |
|------------------------------------------------------------|----|----|----|----|----|----|----|----|----|---------------------------|----|----|----|----|--|--|--|--|--|-----|-----|--|--|--|
| -----FR2----- | | | | | | | | | | Sites of variegation-><2> | | | | | | | | | | <2> | <3> | | | |
| ----->CDR2..... ----- | | | | | | | | | | | | | | | | | | | | <2> | <3> | | | |
| 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | | | | | | | | | | |
| Q A P G K G L E W V S - I - - | | | | | | | | | | | | | | | | | | | | <2> | <3> | | | |
| caa gct cct ggt aaa ggt ttg gag tgg ggt tct - atc - - | | | | | | | | | | | | | | | | | | | | <2> | <3> | | | |
| ...BstXI | | | | | | | | | | | | | | | | | | | | <2> | <3> | | | |

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<1>          <1> 25992-fold diversity in CDR2
.....CDR2.....|-----FR3-----
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
S  G  G  -  T  -  Y  A  D  S  V  K  G  R  F
|tct|ggt|ggc| - |act| - |tat|gct|gac|tcc|ggt|aaa|ggt|cgc|ttc|

-----FR3-----
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T  I  S  R  D  N  S  K  N  T  L  Y  L  Q  M
|act|atc|tct|aga|gac|aac|tct|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
XbaI

-----FR3----->|
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
N  S  L  R  A  E  D  T  A  V  Y  Y  C  A  K
|aac|agc|tta|agg|gct|gag|gac|acc|gct|gtc|tac|tac|tgc|gcc|aaa|
AflII

.....CDR3.....| Replaced by the various components!
121 122 123 124 125 126 127
D  Y  E  G  T  G  Y
|gac|tat|gaa|ggt|act|ggt|tat|

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(SEQ ID NOS: 25-26 are nucleotide and amino acid sequence, respectively)

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|----- FR4 ---(JH4)-----  
Y F D Y W G Q G T L V T V S S  
|tat|ttc|gat|tat|tgg|ggt|caa|ggt|acc|ctg|gtc|acc|gtc|tct|agt|...  
KpnI BstEII
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Table 2: Oligonucleotides used to variegate CDR1 of human HC

CDR1 - 5 residues

(ON-R1V1vg): 5'-ct|tcc|gga|ttc|act|ttc|tct|<1>|tac|<1>|atg|<1>|tgg|gtt|cgc|caa|gct|cct|gg-3'
(SEQ ID NO: 27)

<1> = Codons of ADEFGHIKLMNPQRSTVWY 1:1

(ON-R1top): 5'-cctactgtct|tcc|gga|ttc|act|ttc|tct-3' (SEQ ID NO: 28)

(ON-R1bot) [RC]: 5'-tgg|gtt|cgc|caa|gct|cct|ggttgctcactc-3' (SEQ ID NO: 29)

CDR1 - 7 residues

(ON-R1V2vg): 5'-ct|tcc|gga|ttc|act|ttc|tct|<6>|<7>|tac|tac|tgg|<7>|tgg|gtt|cgc|caa|gct|
cct|gg-3' (SEQ ID NO: 30)

<6> = Codons for ST, 1:1

<7> = 0.2025(Codons for SG) + 0.035(Codons for ADEFGHIKLMNPQRTVWY)

CDR1 - 14 residues

(ON-R1V3vg): 5'-ct|tcc|gga|ttc|act|ttc|tct|atc|agc|ggt|tct|atc|tcc|<1>|<1>|<1>|
tac|tac|tgg|<1>|tgg|gtt|cgc|caa|gct|cct|gg-3' (SEQ ID NO: 31)

<1> = Codons for ADEFGHIKLMNPQRSTVWY 1:1

Table 3: Oligonucleotides used to variegate CDR2 of human HC

| | |
|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| CDR2 - 17 residues | |
| (ON-R2V1vg): | 5'-ggg ttg gag tgg gtt tct <2> atc <2> <3> tct ggg ggc <1> act <1> tat gct - gac tcc gtt aaa gg-3' (<u>SEQ ID NO: 32</u>) |
| (ON-R2top): | 5'-ct tgg gtt cgc caa gct cct ggg aaa ggg tgg gag tct-3' (<u>SEQ ID NO: 33</u>) |
| (ON-R2bot) [RC]: | 5'-tat gct gac tcc gtt aaa ggg cgc ttc act atc tct aga ttcctgtcac-3' (<u>SEQ ID NO: 34</u>) |
| <1> = Codons for A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W and Y (equimolar mixture) | |
| <2> = Codons for Y,R,W,V,G and S (equimolar mixture) | |
| <3> = Codons for P and S (equimolar mixture) or P,S and G (equimolar mixture) | |
| (ON-R2V2vg): | 5'-ggg ttg gag tgg gtt tct <1> atc <4> <1> <1> ggg <5> <1> <1> tat gct - gac tcc gtt aaa gg-3' (<u>SEQ ID NO: 35</u>) |
| <4> = Codons for DINSWY (equimolar mixture) | |
| <5> = Codons for SGDN, (equimolar mixture) | |

CDR2 - 16 residues

(ON-R2V3vg): 5'-ggg|ttg|gag|tgg|ggt|tct|<1>|atc|<4>|<1>|<1>|ggg|
<5>|<1>|<1>|tat|aac|cct|tcc|ctt|aag|gg-3' (SEQ ID NO: 36)

(ON-R2bo3) [RC]: 5'-tat|aac|cct|tcc|ctt|aag|ggg|cgc|ttc|act|atc|tct|aga|ttcctgtcac-3' (SEQ ID
NO: 37)

CDR2 - 19 residues

(ON-R2V4vg): 5'-ggg|ttg|gag|tgg|ggt|tct|<1>|atc|<8>|agt|<1>|<1>|
<1>|ggg|ggg|act|act|act|<1>|tat|gcc|gct|tcc|ggt|aag|gg-3' (SEQ ID NO: 38)

(ON-R2bo4) [RC]: 5'-tat|gcc|gct|tcc|ggt|aag|ggg|cgc|ttc|act|atc|tct|aga|ttcctgtcac-3' (SEQ ID
NO: 39)

<1>, <2>, <3>, <4> and <5> are as defined above

<8> is 0.27 R and 0.027 each of ADEFGHIKLMNPQSTVWY

Table 4: Preferred Components of HC CDR3
(SEQ ID NOS 6-13, respectively, in order of appearance)

| <u>Component</u> | <u>Length</u> | <u>Complexity</u> | <u>Fraction of</u> | | <u>Fraction</u> |
|------------------|-----------------------------------------------------------------------------------------------------|----------------------------|--------------------|-----------------|-----------------|
| | | | <u>Library</u> | <u>Adjusted</u> | |
| 1 | YYCA21111YFDYWG. 8 (1=any amino acid residue, except C; 2 = K and R) | 2.6 x 10 ⁵ .10 | | | .02 |
| 2 | YYCA211111YFDYWG. 10 (1=any amino acid residue, except C; 2 = K and R) | 9.4 x 10 ⁷ .14 | | | .14 |
| 3 | YYCA21111111YFDYWG. 12 (1=any amino acid residue, except C; 2 = K and R) | 3.4 x 10 ¹⁰ .25 | | | .25 |
| 4 | YYCAR111S2S3111YFDYWG. 14 (1=any amino acid residue, except C; 2 = S and G 3 = Y and W) | 1.9 x 10 ⁸ .13 | | | .14 |
| 5 | YYCA21111CSG11CY1YFDYWG. 15 (1=any amino acid residue, except C; 2 = K and R) | 9.4 x 10 ⁷ .13 | | | .14 |
| 6 | YYCA211S1TIFG1111YFDYWG. 17 (1=any amino acid residue, except C; 2 = K and R) | 1.7 x 10 ¹⁰ .11 | | | .12 |
| 7 | YYCAR111YY2S33YY111YFDYWG. 18 (1=any amino acid residue, except C; 2 = D or G; 3 = S and G) | 3.8 x 10 ⁸ .04 | | | .08 |
| 8 | YYCAR1111YC2231CY111YFDYWG. 19 (1=any amino acid residue, except C; 2 = S and G; 3 = T, D and G) | 2.0 x 10 ¹¹ .10 | | | .11 |

Component 2

(C2t10):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|<1>|<1>|<1>|<1>|<1>|<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3'
(SEQ ID NO: 45)

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 3

(C3t12):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|<1>|<1>|<1>|<1>|<1>|<1>|tac|ttc|gat|tac|-
tgg|ggc|caa|gg-3' (SEQ ID NO: 46)

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 4

(C4t140):

5'-cc|gct|gtc|tac|tac|tgc|gcc|cgt|<1>|<1>|<1>|tct|<2>|tct|<3>|<1>|<1>|tac|ttc|gat|-
tac|tgg|ggc|caa|gg-3' (SEQ ID NO: 47)

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = S and G (equimolar mixture); <3> = Y and W (equimolar mixture)

Component 5

(C5t15):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|<1>|tgc|tct|ggc|<1>|<1>|tgc|tat|<1>|tac|-
ttc|gat|tac|tgg|ggc|caa|gg-3' (SEQ ID NO: 48)

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 6

(C6t17):

5'-cc|gct|gct|tac|tac|tgc|tgc|gcc|<2>|<1>|<1>|tct|<1>|act|atc|ttc|ggt|<1>|<1>|<1>|-
<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3' (SEQ ID NO: 49)

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 7

(C7t18):

5'-cc|gct|gct|tac|tac|tgc|tgc|cgt|<1>|<1>|<1>|tat|tac|<2>|tct|<3>|<3>|tac|tat|-
<1>|<1>|<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3' (SEQ ID NO: 50)

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = D and G (equimolar mixture); <3> = S and G (equimolar mixture)

Component 8

(c8t19):

5'-cc|gct|gtc|tac|tac|tgc|gcc|cgt|<1>|<1>|<1>|<1>|tat|tgc|<2>|<2>|<3>|<1>|tgc|tat|-
<1>|<1>|<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3' (SEQ ID NO: 51)

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = S and G (equimolar mixture); <3> = TDG (equimolar mixture);

Table 6: 3-23::JH4 Stuffers in place of CDRs
 (SEQ ID NOS 52-53 are nucleotide and encoded a.a. sequence, respectively)

```

FR1 (DP47/V3-23) -----
      20 21 22      23 24 25 26 27 28 29 30
      A M A      E V Q L L E S G
ctgtctgaac(SEQ ID NO: 99) cc atg gcc      gaa|gtt|caa|ttg|tta|gag|tct|ggt|
Scab..... NcoI.... MfeI

```

```

-----FR1-----
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
      G G L V Q P G G S L R L S C A
|ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|

```

```

-----FR1----->|...CDR1 stuffer....|----FR2-----
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
      A S G F T F S S Y A | | W V R
|gct|tcc|gga|ttc|act|ttc|tct|tcg|tac|gct|tag|taa|tgg|ggt|cgc|
      BspEI          BsiWI          BstXI.

```

```

-----FR2----->|...CDR2 stuffer.
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
Q A P G K G L E W V S | p r |
|caa|gct|cct|ggt|aaa|ggt|ttg|gag|tgg|ggt|tct|taa|cct|agg|tag|
...BstXI
AvrII..
.....CDR2 stuffer.....|---FR3---
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T I S R D N S K N T L Y L Q M
|act|atc|tct|aga|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
XbaI
----FR3-----> CDR3 Stuffer----->|
106 107 108 109 110
N S L R A
|aac|agc|tta|agg|gct|tag|taa|agg|cct|taa
AflII
StuI...

```

(SEQ ID NOS 25-26 are nucleotide and encoded a.a. sequence, respectively)

```
|----- FR4 --- (JH4) -----  
Y F D Y W G Q G T L V T V S S  
|tat|ttc|gat|tat|tgg|ggt|caa|ggt|acc|ctg|gtc|acc|gtc|tct|agt|...  
KpnI BstEII
```

Table 7: A27:JH1 Human Kappa light chain gene
(SEQ ID NOS 54-55 are nucleotide and encoded a.a. sequence, respectively)

| | |
|---------------------------------------------------------------------|----------------------------------|
| gaggacc attggggccc ctccgagact ctcgagcgca | |
| Scab..... EcoO109I | XhoI.. |
| ApaI. | |
| acgcaattaa tgtgagtttag ctcaactcatt aggcacccca ggctttacac tttatgcttc | |
| ...-35.. | Plac ..-10. |
| cggctcgat gttgtgtgga attgtgagcg gataacaatt tcacacagga | |
| aacagctatg accatgatta | |
| cgccaagctt tggagccttt tttttggaga ttttcaac | |
| Pf1MI..... | |
| Hind III | |

M13 III signal sequence (AA seq)----->

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
M K K L L F A I P L V V P F Y
gtg aag aag ctc cta ttt gct atc ccg ctt gtc gtt ccg ttt tac

--Signal-->FR1----->

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
S H S A Q S V L T Q S P G T L
|agc|cat|agt|gca|caa|tcc|gtc|ctt|act|caa|tct|cct|ggc|act|ctt|
ApaLI...

----- FR1 ----->| CDR1----->

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
S L S P G E R A T L S C R A S
|tcg|cta|agc|ccg|ggg|gaa|cgt|gct|acc|tta|agt|tgc|cgt|gct|tcc|
EspI..... AflII....

XmaI.....

For CDR1:

- <1> ADEFGHIKLMNPQRSTVWY 1:1
- <2> S(0.2) ADEFGHIKLMNPQRTVWY (0.044 each)
- <3> Y(0.2) ADEFGHIKLMNPQRSTVW (0.044 each)

(CDR1 installed as AflII-(SexAI or Kasi) cassette.) For the most preferred 11 length codon 51 (XXX) is omitted; for the preferred 12 length this codon is <2>

```
----- CDR1 ----->|--- FR2 ----->
<1>      <2> <2> xxx <3>
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
Q  -  V  -  -  -  -  L  A  W  Y  Q  Q  K  P
|cag| - |gtt| - | - | - | - |ctt|gct|tgg|tat|caa|cag|aaa|cct|
SexAI...
```


For CDR2:

- <1> ADEFGHIKLMNPQRSTVWY 1:1
- <2> S(0.2) ADEFGHIKLMNPQRTVWY (0.044 each)
- <4> A(0.2) DEFGHIKLMNPQRSTVWY (0.044 each)

CDR2 installed as (SexAI or Kasi) to (BamHI or RsrII) cassette.)

```
----- FR2 ----->|----- CDR2 ----->
                                     <1>      <2>      <4>
61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
   G   Q   A   P   R   L   L   I   Y   -   A   S   -   R   -
|ggt|cag|gcg|ccg|cgt|tta|ctt|att|tat| - |gct|tct| - |cgc| - |
SexAI.... Kasi....
```

CDR2-->|--- FR3 ----->

<1>

76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
- G I P D R F S G S G S G T D
| - |ggg|atc|ccg|gac|cgt|tct|tct|ggc|tct|ggg|tca|ggg|act|gac|

BamHI...

RsrII.....

----- FR3 ----->

91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
F T L T I S R L E P E D F A V
|ttt|acc|ctt|act|att|tct|aga|ttg|gaa|cct|gaa|gac|ttc|gct|gtt|

XbaI...

For CDR3 (Length 9):

<1> ADEFGHIKLMNPQRSTVWY 1:1

<3> Y(0.2) ADEFGHIKLMNPQRTVW (0.044 each)

For CDR3 (Length 8): QQ33111P

1 and 3 as defined for Length 9

For CDR3 (Length 10): QQ3211PP1T (SEQ ID NO: 17)

1 and 3 as defined for Length 9

2 S(0.2) and 0.044 each of ADEFGHIKLMNPQRTVWY

CDR3 installed as XbaI to (StyI or BsiWI) cassette.

```
----->|-----CDR3----->|-----FR4----->
      <3> <1> <1> <1> <1> <1>
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
    Y  Y  C  Q  Q  -  -  -  -  -  P  -  T  F  G  Q
|tat|tat|tgc|caa|cag| - | - | - | - |cct| - |act|ttc|ggt|caa|
      BstXI.....
```

```

-----FR4----->| <----- Ckappa -----
121 122 123 124 125 126 127      128 129 130 131 132 133 134
    G  T  K  V  E  I  K          R  T  V  A  A  P  S
|ggg|acc|aag|gtt|gaa|atc|aag|   |cgt|acg|gtt|gcc|gct|cct|agt|
    StyI....                      BsiWI...

```

```

135 136 137 138 139 140 141 142 143 144 145 146 147 148 149
    V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T
|gtg|ttt|atc|ttt|cct|cct|tct|gac|gaa|caa|ttg|aag|tca|ggt|act|
                                MfeI...

```

```

150 151 152 153 154 155 156 157 158 159 160 161 162 163 164
    A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A
|gct|tct|gtc|gta|tgt|ttg|ctc|aac|aat|ttc|tac|cct|cgt|gaa|gct|
                                BssSI...

```

165 166 167 168 169 170 171 172 173 174 175 176 177 178 179
K V Q W K V D N A L Q S G N S
|aaa|gtt|cag|tgg|aaa|gtc|gat|aac|gcg|ttg|cag|tcg|ggt|aac|agt|

MluI....

180 181 182 183 184 185 186 187 188 189 190 191 192 193 194
Q E S V T E Q D S K D S T Y S
|caa|gaa|tcc|gtc|act|gaa|cag|gat|agt|aag|gac|tct|acc|tac|tct|

195 196 197 198 199 200 201 202 203 204 205 206 207 208 209
L S S T L T L S K A D Y E K H
|ttg|tcc|tct|act|ctt|act|tta|tca|aag|gct|gat|tat|gag|aag|cat|

210 211 212 213 214 215 216 217 218 219 220 221 222 223 224
K V Y A C E V T H Q G L S S P
|aag|gtc|tat|Gct|Tgc|gaa|ggt|acc|cac|cag|ggt|ctg|agc|tcc|cct|

SacI....

225 226 227 228 229 230 231 232 233 234
V T K S F N R G E C . .
|gtt|acc|aaa|agt|ttc|aac|cgt|cgt|ggt|gaa|tgc|taa|tag|ggc|gccc
DsaI....
AsclI....
BssHII

acgcattctctaa gcggccgc aacaggaggag
NotI....

Table 8: 2a2:JH2 Human lambda-chain gene
 (SEQ ID NOS 56-57 are nucleotide and encoded a.a. sequence, respectively)

gaggaccatt gggcccc ttactccgtgac
 Scab..... EcoO109I
 ApaI..

| | | | | | | | | | | | | | | | | |
|---------------------------------------------------------------------|---|---|---|---|---------|---|---|---|----|-----------|----|----|----|----|---|---|
| -----FR1-----> | | | | | | | | | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | | |
| S | A | Q | S | A | L | T | Q | P | A | S | V | S | G | S | P | G |
| agt gca caa tcc gct ctc act cag cct gct agc gtt tcc ggg tca cct ggt | | | | | | | | | | | | | | | | |
| ApaLI... | | | | | NheI... | | | | | BstEII... | | | | | | |
| SexAI.... | | | | | | | | | | | | | | | | |

For CDR1 (length 14):
 <1> = 0.27 T, 0.27 G, 0.027 each of ADEFHIKLMNPQRSVWY, no C
 <2> = 0.27 D, 0.27 N, 0.027 each of AEFGHIKLMNPQSTVWY, no C
 <3> = 0.36 Y, 0.0355 each of ADEFGHIKLMNPQSTVW, no C

```

-----FR1-----> |-----CDR1-----
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Q S I T I S C T G - S S - V G
|caa|agt|atc|act|att|tct|tgt|aca|ggg|-|tct|tct|-|gtt|ggc|
BsrGI..

<1> <3> <2> <3> V S = vg Scheme #1, length = 14
-----CDR1-----> |-----FR2-----
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
- - - - - V S W Y Q Q H P G K A
| - | - | - | - |gtt|tct|tgg|tat|caa|caa|cac|ccg|ggc|aag|gcg|
XmaI.... KasI.....
AvaI.....

```

A second Vg scheme for CDR1 gives segments of length 11:

T₂₂G<2><4>L<4><4><3><4><4> where

<4> = equimolar mixture of each of ADEFGHIKLMNPQRSTVWY, no C

<3> = as defined above for the alternative CDR1


```

For CDR2:
<2> and <4> are the same variegation as for CDR1
      <4> <4> <4> <2> R P S
--FR2-----> |-----CDR2----->|-----FR3--
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
P K L M I Y - - - - R P S G V
|ccg|aag|ttg|atg|atc|tac| - | - | - |cgt|cct|tct|ggt|ggt|
KasI....

-----FR3-----
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
S N R F S G S K S G N T A S L
|agc|aat|cgt|ttc|tcc|gga|tct|aaa|tcc|ggt|aat|acc|gca|agc|tta|
BspEI...
HindIII.
BsaBI.....(blunt)

```

```

-----FR3----->|
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
T I S G L Q A E D E A D Y Y C
|act|atc|tct|ggt|ctg|cag|gct|gaa|gac|gag|gct|gac|tac|tat|tgt|
PstI...

```

CDR3 (Length 11):

<2> and <4> are the same variegation as for CDR1

<5> = 0.36 S, 0.0355 each of ADEFGHIKLMNPQRTVWY no C

CDR3 (Length 10): <5> SY <1> <5> S <5> <1> <4> V (SEQ ID NO: 19)

<1> is an equimolar mixture of ADEFGHIKLMNPQRTVWY, no C

<4> and <5> are as defined for Length 11

<4> <5> <4> <2> <4> S <4> <4> <4> <4> V

```

-----CDR3----->|-----FR4-----
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
- - - - - S - - - - V F G G G
| - | - | - | - | tct| - | - | - | gtc|ttc|ggc|ggg|ggg|
KpnI...

-----FR4----->
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
T K L T V L G Q P K A A P S V
|acc|aaa|ctt|act|gtc|ctc|ggg|caa|cct|aag|gct|gct|cct|tcc|gtt|
KpnI...
HincII...
Bsu36I...

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
T L F P P S S E E L Q A N K A
|act|ctc|ttc|cct|cct|agt|tct|gaa|gag|ctt|caa|gct|aac|aac|gct|
SapI.....

```

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
T L V C L I S D F Y P G A V T
|act|ctt|gtt|tgc|ttg|atc|agt|gac|ttt|tat|cct|ggt|gct|gtt|act|

BclI....

151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
V A W K A D S S P V K A G V E
|gtc|gct|tgg|aaa|gcc|gat|tct|tct|cct|gtt|aaa|gct|ggt|gtt|gag|

BsmBI....

166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
T T T P S K Q S N N K Y A A S
|acg|acc|act|cct|tct|aaa|caa|tct|aac|aat|aag|tac|gct|gcg|agc|

BsmBI....

SacI....

181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
S Y L S L T P E Q W K S H K S
|tct|tat|ctt|tct|ctc|acc|cct|gaa|caa|tgg|aag|tct|cat|aaa|tcc|

SacI...

196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
Y S C Q V T H E G S T V E K T
|tat|tcc|tgt|caa|ggt|act|cat|gaa|ggt|tct|acc|ggt|gaa|aag|act|

BspHI...

211 212 213 214 215 216 217 218 219

V A P T E C S . .

|ggt|gcc|cct|act|gag|tgt|tct|tag|tga|ggcgcgcc

Ascl....

BssHII

aacgatgttc aag gcggccgc aacaggaggag

NotI.... Scab.....

Table 9: Oligonucleotides For Kappa and Lambda Light Chain Variegation

| | |
|----------------------------------------------------------------------------------------------------------------------|-----------|
| (Ctop25): 5'-gctctggtcaac tta agg gct gag g-3' (<u>SEQ ID NO: 58</u>) | |
| (CtprmA): 5'-gctctggtcaac tta agg gct gag gac acc gct gtc tac tac tgc gcc-3' (<u>SEQ ID NO: 59</u>) | |
| AflII... | |
| (CBprmB) [RC]: 5'- tac ttc gat tac ttg ggc caa ggg acc ctg gtc acc tcgctccacc-3' (<u>SEQ ID NO: 60</u>) | BstEII... |
| (CBot25) [RC]: 5'- ggg acc ctg gtc acc tcgctccacc-3' (<u>SEQ ID NO: 61</u>) | |
| Kappa chains: CDR1 ("1"), CDR2 ("2"), CDR3 ("3") | |
| CDR1 | |
| (Ka1Top610): 5'-ggctctcagttg cta agc ccg ggg gaa cgt gct acc tta agt tgc cgt gct tcc cag-3' (<u>SEQ ID NO: 62</u>) | |
| (Ka1STp615): 5'-ggctctcagttg cta agc ccg ggg g-3' (<u>SEQ ID NO: 63</u>) | |
| (Ka1Bot620) [RC]: 5'-ctt gct tgg tat caa cag aaa cct ggg cag gcg ccaagtcgtgtc-3' (<u>SEQ ID NO: 64</u>) | |
| (Ka1SB625) [RC]: 5'-cct ggg cag gcg ccaagtcgtgtc-3' (<u>SEQ ID NO: 65</u>) | |

(Ka1vg600): 5'-gct|acc|tta|agt|tgc|cgt|gct|tcc|cag-
 |<1>|gtt|<2>|<2>|<3>|ctt|gct|tgg|tat|caa|cag|aaa|cc-3' (SEQ ID NO: 66)

(Ka1vg600-12): 5'-gct|acc|tta|agt|tgc|cgt|gct|tcc|cag-
 |<1>|gtt|<2>|<2>|<3>|ctt|gct|tgg|tat|caa|cag|aaa|cc-3' (SEQ ID NO: 67)

CDR2

(Ka2Tshort657): 5'-cacgagtccta|cct|ggg|cag|gc-3' (SEQ ID NO: 68)

(Ka2Tlong655): 5'-cacgagtccta|cct|ggg|cag|gcg|ccg|cgt|tta|ctt|att|tat-3' (SEQ ID NO: 69)

(Ka2Bshort660): [RC]: 5'-|gac|cgt|ttc|tct|ggg|tctcacc-3' (SEQ ID NO: 70)

(Ka2vg650): 5'-cag|gcg|ccg|cgt|tta|ctt|att|tat|<1>|gct|tct|<2>|-
 |cgc|<4>|<1>|ggg|atc|ccg|gac|cgt|ttc|tct|ggg|tctcacc-3' (SEQ ID NO: 71)

CDR3

(Ka3Tlon672): 5'-gacgagtccttct|aga|ttg|gaa|cct|gaa|gac|ttc|gct|gct|tat|tgc|caa|c-3'
 (SEQ ID NO: 72)

(Ka3BotL682) [RC]: 5'-act|ttc|ggg|caa|ggg|acc|aag|ggt|gaa|atc|aag|cgt|acg|tcacagggtgag-3'
 (SEQ ID NO: 73)

(Ka3Bsho694) [RC]: 5'-gaa|atc|aag|cgt|acg|tcacagggtgag-3' (SEQ ID NO: 74)

(Ka3vg670): 5'-gac|ttc|gct|ggt|-
|tat|tat|tgc|caa|cag|<3>|<1>|<1>|<1>|cct|<1>|act|ttc|ggt|caa|-
|ggt|acc|aag|ggt|g-3' (SEQ ID NO: 75)

(Ka3vg670-8): 5'-gac|ttc|gct|ggt|-
|tat|tat|tgc|caa|cag|<3>|<3>|<1>|<1>|cct|ttc|ggt|caa|-
|ggt|acc|aag|ggt|g-3' (SEQ ID NO: 76)

(Ka3vg670-10): 5'-gac|ttc|gct|ggt|tat|-
|tat|tgc|caa|cag|<3>|<2>|<1>|<1>|cct|cct|<1>|act|ttc|ggt|caa|-
|ggt|acc|aag|ggt|g-3' (SEQ ID NO: 77)

Lambda Chains: CDR1 ("1"), CDR2 ("2"), CDR3 ("3")

CDR1

(Lm1TPri75): 5'-gacgagtcctgg|tca|cct|ggt|-3' (SEQ ID NO: 78)
(Lm1tlo715): 5'-gacgagtcctgg|tca|cct|ggt|caa|agt|atc|act|att|tct|tgt|aca|ggt-3' (SEQ ID NO: 79)
(Lm1blo724) [rc]: 5'-gtt|tct|tgg|tat|caa|caa|cac|ccg|ggc|aag|gcg|agatcttcacaggtgag-3' (SEQ ID

NO: 80)

(Lm1bsh737) [rc]: 5'-gc|aag|gcg|agatcttcacaggtgag-3' (SEQ ID NO: 81)
(Lm1vg710b): 5'-gt|atc|act|att|tct|tgt|aca|ggt|<2>|<4>|ctc|<4>|<4>|<4>|<3>|<4>|<4>|tgg|tat|caa|cac|cc-3' (SEQ ID NO: 82)

(Lm1vg710): 5'-gt|atc|act|att|tct|tgt|aca|ggg|<1>|tct|tct|<2>|gtt|ggc| -
|<1>|<3>|<2>|<3>|gtt|tct|tgg|tat|caa|caa|cac|cc-3' (SEQ ID NO: 83)

CDR2

(Lm2TSh757): 5'-gagcagaggac|ccg|ggc|aag|gc-3' (SEQ ID NO: 84)
(Lm2TL0753): 5'-gagcagaggac|ccg|ggc|aag|gcg|ccg|aag|ttg|atg|atc|tac|-3' (SEQ ID NO: 85)
(Lm2BL0762) [RC]: 5'-cgt|cct|tct|ggg|gtc|agc|aat|cgt|ttc|tcc|gga|tcacaggtgag-3' (SEQ ID NO: 86)
(Lm2BSh765) [RC]: 5'-cgt|ttc|tcc|gga|tcacaggtgag-3' (SEQ ID NO: 87)
(Lm2vg750): 5'-g|ccg|aag|ttg|atg|atc|tac| -
<4>|<4>|<4>|<2>|cgt|cct|tct|ggg|gtc|agc|aat|c-3' (SEQ ID NO: 88)

CDR3

(Lm3TSh822): 5'-ctg|cag|gct|gaa|gac|gag|gct|gac-3' (SEQ ID NO: 89)
(Lm3TL0819): 5'-ctg|cag|gct|gaa|gac|gag|gct|gac|tac|tat|tgt|-3' (SEQ ID NO: 90)
(Lm3BL0825) [RC]: 5'-gtc|ttc|ggc|ggg|ggg|acc|aaa|ctt|act|gtc|ctc|ggg|caa|cct|aag|g-
acacaggtgag-3' (SEQ ID NO: 91)
(Lm3BSh832) [RC]: 5'-c|ggg|caa|cct|aag|gacacaggtgag-3' (SEQ ID NO: 92)

(Lm3vg817): 5'-gac|gag|gct|gac|tac|tat|tgt|-
 |<4>|<5>|<4>|<2>|<4>|tct|<4>|<4>|<4>|<4>|-
 Gtc|ttc|ggc|ggg|ggg|acc|aaa|ctt|ac-3' (SEQ ID NO: 93)

(Lm3vg817-10): 5'- gac|gag|gct|gac|tac|tat|tgt|-
 |<5>|agc|tat|<1>|<5>|tct|<5>|<1>|<4>|gtc|ttc|ggc|ggg|ggg|-
 |acc|aaa|ctt|ac-3' (SEQ ID NO: 94)

Table 10: A27:JH1 Kappa light chain gene with stuffers in place of CDRs

(SEQ ID NOS 95-96 are nucleotide and encoded a.a. sequence, respectively)

Each stuffer contains at least one stop codon and a restriction site that will be unique within the diversity vector.

gaggacc attggggccc ctccgagact ctcgagcgca

Scab.....EcoO109I

ApaI.

XhoI..

acgcaattaa tgtgagtttag ctcaactcatt aggcacccca ggctttacac tttatgcttc

...-35..

Plac

...-10.

cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacacagga aacagctatgac

catgatta cgccaagctt tggagccttt tttttggaga ttttcaac

PflMI.....

Hind3.

M13 III signal sequence (AA seq)----->

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
M K K L L F A I P L V V P F Y
gtg aag aag ctc cta ttt gct atc ccg ctt gtc gtt ccg ttt tac

--Signal--> FR1----->

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
S H S A Q S S V L T Q S P G T L
|agc|cat|agt|gca|caa|tcc|gtc|ctt|act|caa|tct|cct|ggc|act|ctt|

ApaLI...

----- FR1 ----->|-----Stuffer-->

31 32 33 34 35 36 37 38 39 40 41 42 43
S L S P G E R A T L S | |
|tcg|cta|agc|ccg|ggg|gaa|cgt|gct|acc|tta|agt|tag|taa|gct|ccc|

EspI.....

AflII...

XmaI.....

```

- Stuffer for CDR1--> FR2 ----- FR2 ----->|-----Stuffer for CDR2
      59 60 61 62 63 64 65 66
      K P G Q A P R
|agg|cct|ctt|tga|tct|g|aaa|cct|ggg|cag|gcg|ccg|cgt|taa|tga|aagcgctaattggccaacagtg
StuI...      SexAI...      Kasi....      AfeI...      MscI...

```

```

Stuffer-->|--- FR3 ----->
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
T G I P D R F S G S G S G T D
|act|ggg|atc|ccg|gac|cgt|ttc|tct|ggc|tct|ggg|tca|ggg|act|gac|
      BamHI...
      RsrII.....

```

```

----- FR3 ----->-----STUFFER for CDR3----->
91 92 93 94 95 96 97
F T L T I S R | |
|ttt|acc|ctt|act|att|tct|aga|taa|tga| gttaac tag acc tacgta acc tag
XbaI... HpaI... SnaBI.

-----CDR3 stuffer----->|-----FR4----->
118 119 120
F G Q
|ttc|ggt|caa|

-----FR4----->| <----- Ckappa ----->
121 122 123 124 125 126 127 128 129 130 131 132 133 134
G T K V E I K R T V A A P S
|ggt|acc|aag|ggt|gaa|atc|aag| |cgt|acg|ggt|gcc|gct|cct|agt|
StyI.... BsiWI..

```

135 136 137 138 139 140 141 142 143 144 145 146 147 148 149
V F I F P P S D E Q L K S G T
|gtg|ttt|atc|ttt|cct|cct|tct|gac|gaa|caa|ttg|aag|tca|ggt|act|
MfeI...

acgcattctctaa gcggccgc aacaggaggag

NotI....

EagI..

Table 11: 2a2:JH2 Human lambda-chain gene with stuffers in place of CDRs

(SEQ ID NOS 97-98 are nucleotide and encoded a.a. sequence, respectively)

gaggaccatt gggcccc ttactccgtgac
 Scab..... EcoO109I
 ApaI..

```

-----FR1----->
1  2  3  4  5  6  7  8  9 10 11 12 13 14 15
S  A  Q  S  A  L  T  Q  P  A  S  V  S  G  S  P  G
agt|gca|caa|tcc|gct|ctc|act|cag|cct|gct|agc|gtt|tcc|ggg|tca|cct|ggg|
ApaI...                               NheI...      BstEII...
                                     SexAI....

```

```

-----FR1-----> |-----stuffer for CDR1-----
16 17 18 19 20 21 22 23
Q  S  I  T  I  S  C  T
|caa|agt|atc|act|att|tct|tgt|aca|tct|tag|tga|ctc
BsrGI..

```



```

-----Stuffer----->-----FR2----->
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
R  S  |  |  P  |
aga tct taa tga ccg tag
BglII
cac|ccg|ggc|aag|gcg|
XmaI.... KasI.....
AvaI....

--|-----Stuffer for CDR2 ----->
P
|ccg|taa|tga|atc|tcg|tac|g
KasI.... BsiWI... ct|ggg|gtt|

-----FR3-----
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
S  N  R  F  S  G  S  K  S  G  N  T  A  S  L
|agc|aat|cgt|ttc|tcc|gga|tct|aaa|tcc|ggg|aat|acc|gca|agc|tta|
BspEI.. HindIII.
BsaBI.....(blunt)

```

```

-----FR3----->|--Stuffer for CDR3----->|
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
T I S G L Q
|act|atc|tct|ggt|ctg|cag|ggt|ctg|tag|ttc|caattg|ctt|tag|tga|ccc
PstI... MfeI..

```

```

-----Stuffer----->|-----FR4----->
103 104 105
G G G
|ggc|ggt|ggt|
KpnI...

```

```

-----FR4----->
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
T K L T V L G Q P K A A P S V
|acc|aaa|ctt|act|gtc|ctc|ggt|caa|cct|aag|gct|gct|cct|tcc|gtt|
KpnI... HincII..
Bsui36I...

```

121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
 T L F P P S S E E L Q A N K A
 |act|ctc|ttc|cct|cct|agt|tct|gaa|gag|ctt|caa|gct|aac|aag|gct|
 SapI.....

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
 T L V C L I S D F Y P G A V T
 |act|ctt|ggt|tgc|ttg|atc|agt|gac|ttt|tat|cct|ggg|gct|gtt|act|
 BclI.....